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(54) Title: <b>CHEMICAL COMPOUNDS HAVING ION CHANNEL BLOCKING ACTIVITY FOR THE TREATMENT OF IMMUNE DYSFUNCTION</b>			
(57) Abstract			
<p>The present invention relates to chemical compounds having inhibitory activity on an intermediate conductance <math>Ca^{2+}</math> activated potassium channel (<math>IK_{Ca}</math>), and the use of such compounds for the treatment or alleviation of diseases or conditions relating to immune dysfunction. Moreover, the invention relates to a method of screening a chemical compound for inhibitory activity on an intermediate conductance <math>Ca^{2+}</math> activated potassium channel (<math>IK_{Ca}</math>).</p>			

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## CHEMICAL COMPOUNDS HAVING ION CHANNEL BLOCKING ACTIVITY FOR THE TREATMENT OF IMMUNE DYSFUNCTION

### TECHNICAL FIELD

5

The present invention relates to chemical compounds having inhibitory activity on an intermediate conductance  $\text{Ca}^{2+}$  activated potassium channel ( $\text{IK}_{\text{Ca}}$ ), and the use of such compounds for the treatment or alleviation of diseases or conditions relating to immune dysfunction.

10

Moreover, the invention relates to a method of screening a chemical compound for inhibitory activity on an intermediate conductance  $\text{Ca}^{2+}$  activated potassium channel ( $\text{IK}_{\text{Ca}}$ ).

### BACKGROUND ART

15

Ion channels are transmembrane proteins, which catalyse the transport of inorganic ions across cell membranes. The ion channels participate in processes as diverse as the generation and timing of action potentials, synaptic transmissions, secretion of hormones, contraction of muscles, etc.

20

Many drugs exert their effects via modulation of ion channels. Examples are anti-epileptic compounds like Phenytoin and Lamotrigine, which block voltage dependent  $\text{Na}^{+}$ -channels in the brain, anti-hypertensive drugs like Nifedipine and Diltiazem, which block voltage dependent  $\text{Ca}^{2+}$ -channels in smooth muscle cells, and stimulators of insulin release like Glibenclamide and Tolbutamide, which block an

25

ATP-regulated  $\text{K}^{+}$ -channel in the pancreas.

There is a large and still growing demand for non-toxic immunoregulating agents for use in relation to e.g. organ transplantation and auto-immune diseases.

Some of the currently used immunosuppressive compounds such as Cyclosporin A and FK506 prevent immunological proliferation by inhibition of the  
30  $\text{Ca}^{2+}$ /calmodulin-dependent Ser/Thr phosphatase calcineurin. The usefulness of this class of compounds is limited by their side effects such as renal dysfunction, arterial hypertension, neurological effects (headache, insomnia, tremors, parasthesias, lethargy), gastrointestinal effects (nausea, vomiting, diarrhoea), and diabetes.

Another class of compounds comprising e.g. Azathioprine and Mizorbine interfere in a cytotoxic manner directly with the DNA-replication process. Although cytotoxicity shows some selectivity towards strongly proliferating cells such as activated T- and B-lymphocytes, complications may follow due to effects on dividing  
5 cells in the entire body, including bone marrow, hair sacs, the skin, testis, ovary and epithelia such as the airways, the intestinal tract, and the thick ascending limb of the loop of Henle's.

A fairly new approach for suppression of immune responses is to interfere with ion channels in the plasma membrane of cells in the immune system, especially  
10 the T- and B-lymphocytes. Upon exposure to antigens by antigen presenting macrophages or to mitogens such as IL-2 or IFN- $\gamma$ , an initial signal in the switching from the resting phase to the proliferating phase is an activation of the phosphoinositide signalling pathway resulting in an increase in the intracellular concentration of  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) due to  $\text{Ca}^{2+}$  release from intracellular stores. A sustained  
15 elevated  $[\text{Ca}^{2+}]_i$  is maintained by an increased passive influx through mitogen regulated, voltage-independent Ca-channels. This increase in  $[\text{Ca}^{2+}]_i$  is vital for the subsequent events leading to cell proliferation and secretion of lymphokines.

In resting T- and B-lymphocytes, the  $[\text{Ca}^{2+}]_i$  is approximately  $10^7$  fold higher outside versus inside the cell, and the membrane potential is negative inside, i.e. there  
20 is an inwardly directed electrochemical  $\text{Ca}^{2+}$  gradient. Thus, when the Ca-channels are activated they conduct  $\text{Ca}^{2+}$  into the cell. However,  $\text{Ca}^{2+}$  influx via the Ca-channels, tends to reduce or even eliminate this gradient, and thus to reduce the influx. Concomitant opening of K-channels keeps the membrane potential negative, and activation of these channels is therefore essential for maintaining a large inwardly  
25 directed, electrochemical driving force for  $\text{Ca}^{2+}$ .

In the presence of blockers of lymphocyte K-channels, the cells depolarise, and thereby the  $\text{Ca}^{2+}$  influx necessary for the activation of the immune response is reduced.

Several types of K-channels have been described in B- and T-lymphocytes  
30 including both voltage-dependent K-channels ( $\text{K}_v$ ), and voltage-independent  $\text{Ca}^{2+}$ -activated K-channels ( $\text{K}_{\text{Ca}}$ ). It is well established, that the  $\text{K}_v$ -channels are activated by the  $\text{Ca}^{2+}$ -induced depolarisation of the lymphocyte, and non-selective blockers of  $\text{K}_v$ -channels are therefore quite effective immunosuppressive agents. However, these

compounds in general have severe side effects due to block of repolarisation in excitable tissue (seizures, myotonic runs, high blood pressure, etc.).

Considerable effort has been made into the development of immunoselective  $K_V$ -blockers. The molecular rationale for this, has been the  
5 observation that T-lymphocytes express homomeric  $K_V1.3$ -channels in contrast to excitable cells, which always express several heteromeric subtypes of the  $K_V$ -channels.

A selective blocker of the  $K_V1.3$ -homomer might therefore be an ideal, relatively non-toxic, immunosuppressive agent. Initial reports from these  
10 pharmacological programs indicate that selective  $K_V1.3$ -blockers are very effective as anti-inflammatory agents. However, the well-known toxicity of non-selective  $K_V$ -blockers has apparently not disappeared. An example is the potent  $K_V1.3$  blocker CP-339,818. This compound is also a potent blocker of  $K_V1.4$ , a cardiac and neuronal A-type K-channel. The side-effect of this compound is predicted to be interference with  
15 the cardiac action potential (long QT-syndrome toxicity) as well as with the action potential repolarisation and after hyperpolarization in neurons.

### SUMMARY OF THE INVENTION

20 A hitherto untested alternative to the block of the voltage-dependent K-channels is a selective inhibition of the  $Ca^{2+}$ -activated K-channels in T- and B-lymphocytes. These channels are directly activated by the increased  $[Ca^{2+}]_i$  which is the primary signal for lymphocyte activation. Further, contrary to  $K_V$ -channels, these channels are voltage-independent, and therefore they do not close upon  
25 hyperpolarization, implicating that they are even more effective than  $K_V$  channels in maintaining a large inward driving force on  $Ca^{2+}$  under conditions of elevated intercellular  $Ca^{2+}$ -concentrations.

Two types of  $Ca^{2+}$ -activated K-channels have been described from lymphocytes: 1) Small-conductance, apamin-sensitive,  $Ca^{2+}$ -activated K-channels  
30 ( $SK_{Ca}$ ) and 2) Intermediate-conductance, inwardly rectifying, Clotrimazole-sensitive,  $Ca^{2+}$ -activated K-channels ( $IK_{Ca}$ ), also referred to as Gardos-channels. Resting T-lymphocytes express both  $SK_{Ca}$  and  $IK_{Ca}$ , whereas B-lymphocytes only express  $IK_{Ca}$ .

Upon activation, prior to cell proliferation, the expression level of  $IK_{Ca}$  increases approximately 30 fold in both T- and B-lymphocytes. The expression levels of both  $K_v1.3$  and  $SK_{Ca}$  remain unchanged, indicating a major role for the  $IK_{Ca}$ -channel in induction of T- and B-cell proliferation. Contrary to the  $SK_{Ca}$ -channels, which are extensively expressed in CNS and heart (measured as mRNA abundance by Northern hybridisation) and in PNS, skeletal muscle, hepatocytes (measured as functional channels by electrophysiology), expression of  $IK_{Ca}$ -channels have never been reported from any excitable tissue. In fact, blood cells such as erythrocytes, monocytes, lymphocytes, endothelial cells, and certain cell-lines with an epithelial ancestry, Ehrlich ascites tumor cells and HeLa cells appear to be the main source of this type of channels.

Furthermore, the very recent cloning of  $IK_{Ca}$  has enabled the demonstration of the mRNA for this gene in several organs including placenta, salivary glands, lung and pancreas. Thus, specific blockers of  $IK_{Ca}$  are likely to be very effective as immunosuppressive agents, and devoid of side effects on excitable tissue. In fact, the  $IK_{Ca}$ -inhibitor Clotrimazole (which is also a blocker of the cytochrome P-450 system) has been extensively used clinically in the systemic treatment of fungal infections. No toxicity related to K-channel blockade has been described.

Accordingly, in its first aspect, the invention relates to the use of a chemical compound having  $IK_{Ca}$  inhibitory activity for the manufacture of a medicament for the treatment or alleviation of diseases, disorders or conditions relating to immune dysfunction.

In another aspect the invention provides a pharmaceutical compositions for use in the treatment or alleviation of diseases, disorders or conditions relating to immune dysfunction, comprising an effective amount of a chemical compound having  $IK_{Ca}$  inhibitory activity.

In yet another aspect the invention provides a method of screening a chemical compound for inhibitory activity on an intermediate conductance  $Ca^{2+}$  activated potassium channel ( $IK_{Ca}$ ), which method comprises the steps of subjecting an  $IK_{Ca}$  containing cell to the action of the chemical compound, and monitoring the membrane potential of the  $IK_{Ca}$  containing cell.

## DETAILED DISCLOSURE OF THE INVENTION

The present invention relates to the use of a chemical compound having  $IK_{Ca}$  inhibitory activity for treatment or alleviation of diseases or conditions relating to immune dysfunction.

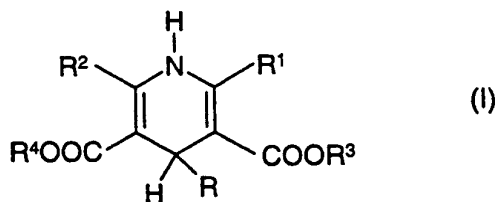
### Chemical Compound having $IK_{Ca}$ Inhibitory Activity

According to the invention, chemical compound having  $IK_{Ca}$  inhibitory activity may be identified by its ability to inhibit hyperpolarization of an  $IK_{Ca}$  containing cell, i.e. a cell containing an intermediate conductance  $Ca^{2+}$  activated potassium channel ( $IK_{Ca}$ ). In a preferred embodiment, the chemical compounds having  $IK_{Ca}$  inhibitory activity is identified by the method of screening described below.

Preferred chemical compounds having  $IK_{Ca}$  inhibitory activity for use according to the invention are the derivatives of 1,4-dihydropyridine-3,5-dicarboxylic acid, the imidazole derivatives, the triazole derivatives, the nitroimidazole derivatives, and the derivatives and metabolites of Clotrimazole, described below. The derivatives of 1,4-dihydropyridine-3,5-dicarboxylic acid have been disclosed in e.g. US 3,799,934. The imidazole derivatives, the triazole derivatives, and the nitroimidazole derivatives have been disclosed in e.g. US 5,273,992. The derivatives and metabolites of Clotrimazole have been disclosed in e.g. WO 96/08242.

### Derivatives of 1,4-dihydropyridine-3,5-dicarboxylic acid

In a preferred embodiment, the chemical compound having  $IK_{Ca}$  inhibitory activity for use according to the invention is a symmetric or asymmetric derivative of 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general formula



wherein

R represents an alkyl group or a cycloalkyl group;

or R represents a mono- or polycyclic aryl group, which aryl group may be substituted one or more times with substituents selected from the group consisting of halogen, trifluoromethyl ( $-\text{CF}_3$ ), nitro ( $-\text{NO}_2$ ), cyano ( $-\text{CN}$ ), azido ( $-\text{N}_3$ ), a group of the formula  $-\text{S}(\text{O})_n\text{-alkyl}$ ,  $-\text{S}(\text{O})_n\text{-NH-alkyl}$ , or  $-\text{S}(\text{O})_n\text{-N-(alkyl)}_2$ , in which n has a value of 0, 1 or 2, an alkyl group, a cycloalkyl group, an alkoxy group, a trifluoromethyl-oxy group ( $-\text{OCF}_3$ ), a carboxy group ( $-\text{COOH}$ ), a group of the formula  $-\text{COO-alkyl}$ , a carbamoyl group ( $-\text{CONH}_2$ ), and a group of the formula  $-\text{CONH-alkyl}$  or  $-\text{CON(alkyl)}_2$ ;

or R represents a mono- or poly-heterocyclic group, which heterocyclic group may be substituted one or more times with alkyl, alkoxy, a carboxy group ( $-\text{COOH}$ ), a group of the formula  $-\text{COO-alkyl}$ , and/or a group of the formula  $-\text{COO-phenyl}$ ;

and  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and  $\text{R}^4$ , independent of each another, represents hydrogen, an alkyl group, a cycloalkyl group, an alkenyl group, an alkynyl group, an alkoxy group, a phenyl group, a phenyl-alkyl group, a furanyl group, a furanyl-alkyl group, a pyridyl group, or a pyridyl-alkyl group;

or a pharmaceutically acceptable acid addition salt thereof.

In a more preferred embodiment, the chemical compound for use according to the invention is a compound of the general formula (I) in which R represents a cyclohexyl group; or R represents a monosubstituted phenyl group, which phenyl group may be substituted one or more times with substituents selected from the group consisting of halogen, trifluoromethyl ( $-\text{CF}_3$ ), nitro ( $-\text{NO}_2$ ), and cyano ( $-\text{CN}$ ); or R represents a pyridyl group or a dihydro-pyridyl group, which groups may be monosubstituted with a group of the formula  $-\text{COO-alkyl}$ , or a group of the formula  $-\text{COO-phenyl}$ .

In a another preferred embodiment, the chemical compound for use according to the invention is a compound of the general formula (I) in which R represents a 2-nitrophenyl group, a 3-nitrophenyl group, a 4-nitrophenyl group, a 2-trifluoromethylphenyl group, a 3-trifluoromethylphenyl group, or a 4-trifluoromethylphenyl group; a 2-cyanophenyl group, a 3-cyanophenyl group, a 4-cyanophenyl group; or R represents a 2-pyridyl, a 3-pyridyl or a 4-pyridyl group, a 1,2-, 1,4- or 1,6-dihydro-2-pyridyl, a 1,2-, 1,4- or 1,6-dihydro-3-pyridyl, or a 1,2- or 1,4-dihydro-4-pyridyl group, which pyridyl or dihydropyridyl groups may be



monosubstituted with C<sub>1-6</sub>-alkyl, a group of the formula -COO-C<sub>1-6</sub>-alkyl, or a group of the formula -COO-phenyl.

In yet another preferred embodiment, the chemical compound for use according to the invention is a compound of the general formula (I) in which R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup>, independent of each another, represents C<sub>1-6</sub>-alkyl, preferably methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

In a more preferred embodiment, the chemical compound for use according to the invention is a compound of the general formula (I) which is an asymmetric derivative of the 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general formula (I). Preferred asymmetric derivatives includes asymmetric C<sub>1-6</sub>-alkyl derivatives of the 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general formula (I). Most preferred asymmetric compounds include

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester (Nitrendipine);

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

- 1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;
- 5 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
10 isopropyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;
- 15 1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid  
20 propyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester;
- 25 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid  
30 ethyl methyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid ethyl  
methyl ester;

5 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid propyl  
methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid isopropyl  
methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid ethyl  
10 methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid propyl  
methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid isopropyl  
methyl ester;

15 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid ethyl  
methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid propyl  
methyl ester; and

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid isopropyl  
20 methyl ester.

In another preferred embodiment, the chemical compound is a symmetric  
derivative of 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general  
formula (I). Preferred chemical compounds include the symmetric C<sub>1-6</sub>-alkyl derivatives  
of the 1,4-dihydropyridine-3,5-dicarboxylic acid. Most preferred symmetric chemical  
25 compounds for use according to the invention include

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester (Nifedipine);

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;

30 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;

- 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;
- 5 1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
10 dimethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;
- 15 1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid  
20 diethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;
- 25 1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid dimethyl  
30 ester;
- 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid diethyl  
ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid diethyl ester;

5 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-cyclohexylpyridine-3,5-dicarboxylic acid dimethyl ester; and

1,4-dihydro-2,6-dimethyl-4-cyclohexylpyridine-3,5-dicarboxylic acid diethyl ester.

#### Imidazole Derivatives

15 In another preferred embodiment, the chemical compound having  $IK_{Ca}$  inhibitory activity for use according to the invention is an imidazole derivative selected from the group consisting of

1-[(2-chlorophenyl)-diphenyl-methyl]-1H-imidazole (Clotrimazole);

1-[(3-chlorophenyl)-diphenyl-methyl]-1H-imidazole;

20 1-[(4-chlorophenyl)-diphenyl-methyl]-1H-imidazole;

1-[(2-chlorophenyl)-(4-hydroxyphenyl)-phenyl-methyl]-1H-imidazole;

1-[(3-chlorophenyl)-(4-hydroxyphenyl)-phenyl-methyl]-1H-imidazole;

1-[(4-chlorophenyl)-(4-hydroxyphenyl)-phenyl-methyl]-1H-imidazole;

1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]-1H-imidazole

25 (Miconazole);

1-Acetyl-4-[4-[(2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)methyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine (Ketoconazole);

1-[2-[(4-chlorophenyl)methoxyl]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole (Econazole);

30 1-[4-(4-chlorophenyl)-2-(2,6-dichlorophenylthio)butyl]imidazole mononitrate (Butoconazole);

2',4'-dichloro-2-imidazol-1-ylacetophenone-(Z)-O-(2,4-dichlorobenzyl)oxime mononitrate (Oxiconazole);

1-[2,4-dichloro- $\beta$ -(4-chlorobenzyl)thiophenethyl]imidazole nitrate  
(Sulconazole); and

1-[2-[(2-chloro-3-thienyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole  
(Thioconazole).

5

### Triazole Derivatives

In a third preferred embodiment, the chemical compound having  $IK_{Ca}$  inhibitory activity for use according to the invention is a triazole derivative selected from the group consisting of

10 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol  
(Fluconazole);

1-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-phenyl]-4-isopropylpiperazine (Terconazole);

15  $(\pm)$ -2-sec-butyl-4-[4-(4-{[(2R\*,4S\*)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-ylmethoxy]phenyl}-piperazin-1-yl)phenyl]-2,4-dihydro-1,2,4-triazol-3-one (Itraconazole).

### Nitroimidazole Derivatives

In a fourth preferred embodiment, the chemical compound having  $IK_{Ca}$  inhibitory activity for use according to the invention is a nitroimidazole derivative selected from the group consisting of

2-methyl-5-nitroimidazole-1-ethanol (Metronidazole);

1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole (Tinidazole);

4-[2-(5-nitroimidazol-1-yl)ethyl]morpholine (Nimorazole);

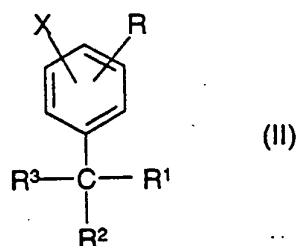
25 1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol (Omidazole), and

N-benzyl-2-(2-nitroimidazol-1-yl)acetamide (Benznidazole).

### Metabolites of Clotrimazole

In yet another preferred embodiment chemical compounds having  $IK_{Ca}$  inhibitory activity for use according to the invention are derivatives and metabolites of Clotrimazole, as described in WO 96/08242.

The derivatives and metabolites of Clotrimazole for use according to the invention may be characterised by the following general formula



wherein

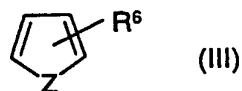
X represents halogen, a trifluoromethyl group, a nitro group, or a cyano group;

5 R represents hydrogen, halogen, hydroxy, an alkyl group, a cycloalkyl group, an alkoxy group, or an alkyloxy group;

R<sup>1</sup> represents hydrogen, or a phenyl group, which phenyl group may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy;

10 R<sup>2</sup> represents hydrogen, hydroxyl, alkyl, alkoxy;

R<sup>3</sup> represents a group of the formula -Y-CH<sub>2</sub>-R<sup>5</sup>, wherein Y represents oxygen (-O-) or sulphur (-S-); a group of the formula =NO-CH<sub>2</sub>R<sup>5</sup>; a group of the formula -O-phenyl-CH=CH<sub>2</sub>; a group of the formula -CH<sub>2</sub>-CH(CH<sub>3</sub>)-S-phenyl, which phenyl may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; or a phenyl group, which phenyl may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; and wherein R<sup>5</sup> represents an ethenyl group (CH<sub>2</sub>=CH-); a phenyl group, which phenyl may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; a phenyl-S-phenyl group, a group of the formula CH<sub>2</sub>-O-phenyl, which phenyl may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; or a group of the formula



wherein Z represents S, O or N;

25 and R<sup>6</sup> represents hydrogen, halogen or hydroxy;  
or a pharmaceutically acceptable acid addition salt thereof.

Preferred derivatives and metabolites for use according to the invention include

- 2-chlorophenyl-4-hydroxyphenyl-phenyl-methane;
- 2-chlorophenyl-bis-phenyl-methane;
- 5 2-chlorophenyl-bis-phenyl-methanol;
- 3-(1-[2,4-dichlorophenyl]-ethoxymethyl)-2-chlorothiophene;
- O-(2,4-dichlorobenzyl)-2,4-dichloroacetophenone oxime;
- 1-(2,4-dichloro)-1-(4-(phenylthio)benzyloxy)ethane;
- 1-(2,4-dichlorophenyl)-1-(allyloxy)ethane;
- 10 1-(2,4-dichlorophenyl)-1-(4-chlorobenzylthio)ethane;
- 1-(2,4-dichlorophenyl)-1-(2,4-dichlorobenzyloxy)ethane;
- 1-(2,4-dichlorophenyl)ethyl-2,6-dichlorobenzyl ether;
- 1-(2-[4-chlorophenoxy]ethyloxy)-1-(2,4-dichlorophenyl)propene;
- 1-(2,4-dichlorophenyl)-ethyl-(4-chlorophenyl)methyl ether;
- 15 3-chlorobenzyl-2-vinylphenyl ether; and
- 1-(4-chlorophenyl)-3-(2,6-dichlorophenylthio)butane.

#### Definition of Substituents

In the context of this invention halogen represents a fluorine, a chlorine, a  
20 bromine or a iodine atom.

In the context of this invention an alkyl group designates a univalent saturated, straight or branched hydrocarbon chain. The hydrocarbon chain preferably contain of from one to eighteen carbon atoms ( $C_{1-18}$ -alkyl), more preferred of from one to six carbon atoms ( $C_{1-6}$ -alkyl; lower alkyl), including pentyl, isopentyl, neopentyl,  
25 tertiary pentyl, hexyl and isohexyl. In a preferred embodiment alkyl represents a  $C_{1-4}$ -alkyl group, including butyl, isobutyl, secondary butyl, and tertiary butyl. In a most preferred embodiment alkyl represents a  $C_{1-3}$ -alkyl group, which may in particular be methyl, ethyl, propyl or isopropyl.

In the context of this invention a cycloalkyl group designates a cyclic alkyl  
30 group, preferably containing of from three to seven carbon atoms ( $C_{3-7}$ -cycloalkyl), including cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

In the context of this invention an alkenyl group designates a carbon chain containing one or more double bonds, including di-enes, tri-enes and poly-enes. In a



preferred embodiment the alkenyl group of the invention comprises of from two to six carbon atoms (C<sub>2-6</sub>-alkenyl), including at least one double bond. In a most preferred embodiment the alkenyl group of the invention is ethenyl; 1,2- or 2,3-propenyl; or 1,2-, 2,3-, or 3,4-butenyl.

5 In the context of this invention an alkynyl group designates a carbon chain containing one or more triple bonds, including di-ynes, tri-ynes and poly-ynes. In a preferred embodiment the alkynyl group of the invention comprises of from two to six carbon atoms (C<sub>2-6</sub>-alkynyl), including at least one triple bond. In its most preferred embodiment the alkynyl group of the invention is ethynyl, 1,2- or 2,3-propynyl, 1,2-,  
10 2,3- or 3,4-butynyl.

In the context of this invention an alkoxy group designates an "alkyl-O-" group, wherein alkyl is as defined above.

In the context of this invention a mono- or polycyclic aryl group designates a monocyclic or polycyclic aromatic hydrocarbon group. Examples of preferred aryl  
15 groups of the invention are phenyl, naphthyl and anthracenyl.

In the context of this invention a mono- or poly-heterocyclic group is a mono- or polycyclic aromatic group, which holds one or more heteroatoms in its ring structure. Preferred heterocyclic monocyclic groups of the invention are 5- and 6 membered heterocyclic monocyclic groups. Examples of preferred heterocyclic  
20 monocyclic groups of the invention include furanyl, imidazolyl, isoimidazolyl, 2-isoimidazolyl, isothiazolyl, isoxazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolyl, thiadiazolyl, thiazolyl, and thienyl. Examples of preferred heterocyclic polycyclic groups of the invention include benzimidazolyl, indolyl,  
25 isoquinolyl and quinolyl.

The chemical compounds for use according to the invention have been described and may be prepared by methods known in the art.

#### Pharmaceutically Acceptable Salts

The chemical compound of the invention may be provided in any form  
30 suitable for the intended administration. Suitable forms include pharmaceutically (i.e. physiologically) acceptable salts, or pre- or prodrug forms of the chemical compound of the invention.

Examples of pharmaceutically acceptable addition salts include, without limitation, the non-toxic inorganic and organic acid addition salts such as the acetate derived from acetic acid, the aconate derived from aconitic acid, the ascorbate derived from ascorbic acid, the benzenesulfonate derived from benzenesulfonic acid, the benzoate derived from benzoic acid, the cinnamate derived from cinnamic acid, the citrate derived from citric acid, the embonate derived from embonic acid, the enantate derived from enanthic acid, the formate derived from formic acid, the fumarate derived from fumaric acid, the glutamate derived from glutamic acid, the glycolate derived from glycolic acid, the hydrochloride derived from hydrochloric acid, the hydrobromide derived from hydrobromic acid, the lactate derived from lactic acid, the maleate derived from maleic acid, the malonate derived from malonic acid, the mandelate derived from mandelic acid, the methanesulfonate derived from methane sulphonic acid, the naphthalene-2-sulphonate derived from naphthalene-2-sulphonic acid, the nitrate derived from nitric acid, the perchlorate derived from perchloric acid, the phosphate derived from phosphoric acid, the phthalate derived from phthalic acid, the salicylate derived from salicylic acid, the sorbate derived from sorbic acid, the stearate derived from stearic acid, the succinate derived from succinic acid, the sulphate derived from sulphuric acid, the tartrate derived from tartaric acid, the toluene-p-sulphonate derived from p-toluene sulfonic acid, and the like. Such salts may be formed by procedures well known and described in the art.

Other acids such as oxalic acid, which may not be considered pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining a chemical compound of the invention and its pharmaceutically acceptable acid addition salt.

Metal salts of a chemical compound of the invention includes alkali metal salts, such as the sodium salt, of a chemical compound of the invention containing a carboxy group.

The chemical compound of the invention may be provided in dissoluble or indissoluble forms together with a pharmaceutically acceptable solvents such as water, ethanol, and the like. Dissoluble forms may also include hydrated forms such as the monohydrate, the dihydrate, the hemihydrate, the trihydrate, the tetrahydrate, and the like. In general, the dissoluble forms are considered equivalent to indissoluble forms for the purposes of this invention.

### Steric Isomers

The chemical compounds of the present invention may exist in (+) and (-) forms as well as in racemic forms. The racemates of these isomers and the individual isomers themselves are within the scope of the present invention.

5 Racemic forms can be resolved into the optical antipodes by known methods and techniques. One way of separating the diastereomeric salts is by use of an optically active acid, and liberating the optically active amine compound by treatment with a base. Another method for resolving racemates into the optical antipodes is based upon chromatography on an optical active matrix. Racemic  
10 compounds of the present invention can thus be resolved into their optical antipodes, e.g., by fractional crystallisation of d- or l- (tartrates, mandelates, or camphorsulphonate) salts for example.

The chemical compounds of the present invention may also be resolved by the formation of diastereomeric amides by reaction of the chemical compounds of the  
15 present invention with an optically active activated carboxylic acid such as that derived from (+) or (-) phenylalanine, (+) or (-) phenylglycine, (+) or (-) camphanic acid or by the formation of diastereomeric carbamates by reaction of the chemical compound of the present invention with an optically active chloroformate or the like.

Additional methods for the resolving the optical isomers are known in the  
20 art. Such methods include those described by *Jaques J, Collet A, & Wilen S* in "Enantiomers, Racemates, and Resolutions", John Wiley and Sons, New York (1981).

Moreover, some of the chemical compounds of the invention being oximes, may thus exist in two forms, syn- and anti-form (Z- and E-form), depending on the arrangement of the substituents around the -C=N- double bond. A chemical compound  
25 of the present invention may thus be the syn- or the anti-form (Z- and E-form), or it may be a mixture hereof.

### **Method of Screening**

In another aspect, the present invention provides a method for the screening  
30 of chemical compounds for inhibitory activity on an intermediate conductance  $\text{Ca}^{2+}$  activated potassium channel ( $\text{IK}_{\text{Ca}}$ ), by which method a chemical compound having  $\text{IK}_{\text{Ca}}$  inhibitory activity is identified by its ability to inhibit hyperpolarization of the cell.

The screening method of the invention comprises the steps of  
subjecting an  $IK_{Ca}$  containing cell to the action of the chemical compound to  
be screened, and

monitoring the membrane potential of the  $IK_{Ca}$  containing cell.

5 More particularly the monitoring of the membrane potential of the  $IK_{Ca}$   
containing cell of step (ii) is carried out in order to monitor changes in the membrane  
potential caused by the action of the chemical compound.

#### The $IK_{Ca}$ Containing Cell

10 The  $IK_{Ca}$  used in the method of the invention may be of any origin, however,  
preferably of human or animal origin. Also, the  $IK_{Ca}$  may be endogenous or it may be  
exogenous to the cell in question.

In a preferred embodiment, the  $IK_{Ca}$  of the  $IK_{Ca}$  containing cell is an ion  
channel that is endogenous to the cell in question, and which cell may in particular be  
15 a T- or B-lymphocyte or other cells known to express  $IK_{Ca}$ , e.g. a HeLa cell, or a cell of  
epithelial origin, a cell of endothelial origin, or a blood cell.

In another preferred embodiment, the  $IK_{Ca}$  of the  $IK_{Ca}$  containing cell is an  
ion channel that is exogenous to the cell in question, and which cell may in particular  
be a human embryonic kidney (HEK) cell, a HEK 293 cell, a Chinese hamster ovary  
20 (CHO) cell, a *Xenopus laevis* oocyte (XLO) cell, or any other cell line able to express  
 $IK_{Ca}$ .

The  $IK_{Ca}$  preferably is of human origin. In particular the  $IK_{Ca}$  may be isolated  
from salivary glands, from lung tissue, from tracheal tissue, from placenta tissue, from  
pancreas tissue, from lymphocytes, from colon tissue, from kidney tissue, from thymus  
25 tissue, from bone marrow, from prostate tissue, from stomach tissue, from liver tissue,  
from foetal liver tissue, from mammary glands, from small intestine tissue, from spleen  
tissue, or from lymph node tissue. Preferably the  $IK_{Ca}$  may be isolated from salivary  
glands, from lung tissue, from tracheal tissue, from placenta tissue, from pancreas  
tissue, or from lymphocytes.

30 In a most preferred embodiment, the  $IK_{Ca}$  is encoded by the DNA sequence  
presented as SEQ ID NO: 1, or a homologous sequence, e.g. a DNA sequence  
showing a homology to SEQ ID NO: 1 of at least 80%, more preferred at least 90%,  
most preferred at least 95%.

### Monitoring of the Membrane Potential

According to the method of the invention the membrane potential is monitored in order to determine changes in the membrane potential. The membrane potential may be monitored using established methods.

In a preferred embodiment monitoring of the membrane potential of the  $IK_{Ca}$  containing cell is performed by patch clamp techniques, e.g. as described by Hamill, O.P., et al., Pflügers Arch. 1981 351 85-100. In a more preferred embodiment, monitoring of the membrane potential of the  $IK_{Ca}$  containing cell is performed by the automatic patch clamp method described in pending patent application DK 1151/97.

In another preferred embodiment monitoring of the membrane potential of the  $IK_{Ca}$  containing cell is performed using fluorescence methods.

In a preferred method of the invention, the  $IK_{Ca}$  containing cell is mixed with a membrane potential indicating agent, that allow for a determination of changes in the membrane potential of the cell, caused by the addition of the test compound.

The membrane potential indicating agent employed in the method of the invention may be any agent that allow monitoring of changes in the membrane potential. In a preferred embodiment, the membrane potential indicating agent is a fluorescent indicator. The fluorescent indicator must be sufficiently sensitive so as to produce a detectable change in fluorescence intensity in the presence of calcium ions.

Preferred fluorescent indicators are in particular DIBAC<sub>4</sub>(3), DiOC5(3), and DiOC2(3).

Monitoring of the membrane potential of the  $IK_{Ca}$  containing cell may then be performed by spectroscopic methods, e.g. using a FLIPR assay (Fluorescence Image Plate Reader; available from Molecular Devices), or by using the automated analysis equipment described in US 5,670,113.

In a separate aspect the invention relates to an encompasses the chemical compounds identified by the method of the invention and their use the use of these compounds for the treatment or alleviation of diseases or conditions relating to immune dysfunction.

### Biological Activity

As described above, the  $IK_{Ca}$  inhibitory compounds of the invention are useful as immune modulating agents, i.e. agents capable of regulating the immune system. More particularly, the  $IK_{Ca}$  inhibitory compounds of the present invention may  
5 be used for reducing or inhibiting undesired immunoregulatory actions.

In a preferred embodiment, the invention relates to the use of an  $IK_{Ca}$  inhibitory compound for the treatment or alleviation of a diseases, disorders or condition related to immune dysfunction.

Conditions which may benefit from this treatment include, but are not limited  
10 to diseases, disorders or conditions such as autoimmune diseases, e.g. Addison's disease, alopecia areata, Ankylosing spondylitis, hemolytic anemia (anemia haemolytica), pernicious anemia (anemia perniosa), aphthae, aphthous stomatitis, arthritis, osteoarthritis, rheumatoid arthritis, aspermiogenesis, asthma bronchiale, autoimmune asthma, autoimmune hemolysis, Bechet's disease, Boeck's disease,  
15 inflammatory bowel disease, Burkitt's lymphoma, Chron's disease, chorioiditis, colitis ulcerosa, Coeliac disease, cryoglobulinemia, dermatitis herpetiformis, dermatomyositis, insulin-dependent type I diabetes, juvenile diabetes, idiopathic diabetes insipidus, insulin-dependent diabetes mellitus, autoimmune demyelinating diseases, Dupuytren's contracture, encephalomyelitis, encephalomyelitis allergica,  
20 endophthalmitis phacoanaphylactica, enteritis allergica, autoimmune enteropathy syndrome, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, glomerulo nephritis, Goodpasture's syndrome, Graves' disease, Hamman-Rich's disease, Hashimoto's disease, Hashimoto's thyroiditis, sudden hearing loss, sensorineural hearing loss, hepatitis chronica, Hodgkin's disease,  
25 haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, iritis, leucopenia, leucemia, lupus erythematosus disseminatus, systemic lupus erythematosus, cutaneous lupus erythematosus, lymphogranuloma malignum, mononucleosis infectiosa, myasthenia gravis, transverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia sympathica, orchitis granulomatosa, pancreatitis, pemphigus,  
30 pemphigus vulgaris, polyarteritis nodosa, polyarthritis chronica primaria, polymyositis, polyradiculitis acuta, psoriasis, purpura, pyoderma gangrenosum, Quervain's thyroiditis, Reiter's syndrome, sarcoidosis, ataxic sclerosis, progressive systemic sclerosis, scleritis, scleroderma, multiple sclerosis, sclerosis disseminata, acquired

spenic atrophy, infertility due to antispermatozoan antibodies, thrombocytopenia, idiopathic thrombocytopenia purpura, thymoma, acute anterior uveitis, vitiligo, AIDS, HIV, SCID and Epstein Barr virus associated diseases such as Sjorgren's syndrome, virus (AIDS or EBV) associated B cell lymphoma, parasitic diseases such as  
5 Lesihmania, and immunosuppressed disease states such as viral infections following allograft transplantations, graft vs. Host syndrome, transplant rejection, or AIDS, cancers, chronic active hepatitis diabetes, toxic chock syndrome, food poisoning, and transplant rejection.

Accordingly, in further embodiments, the invention relates to a chemical  
10 compound having  $IK_{Ca}$  inhibitory activity for use as a medicament.

More specifically the invention relates to the use of a chemical compound having  $IK_{Ca}$  inhibitory activity for use in the manufacture of a medicament for the treatment of treatment of diseases related to immune dysfunction. In a preferred embodiment the medicament is an immune system suppressing medicament (an  
15 immunosuppressivum).

### Pharmaceutical Compositions

In yet another aspect the invention relates to pharmaceutical compositions for use in the treatment or alleviation of diseases, disorders or conditions related to  
20 immune dysfunction, which pharmaceutical composition comprises a therapeutically effective amount of a chemical compound having  $IK_{Ca}$  inhibitory activity, as identified by the method of the invention.

While a chemical compound of the invention for use in therapy may be administered in the form of the raw chemical compound, it is preferred to introduce the  
25 active ingredient, optionally in the form of a physiologically acceptable salt, in a pharmaceutical composition together with one or more adjuvants, excipients, carriers, buffers, diluents, and/or other customary pharmaceutical auxiliaries.

In a preferred embodiment, the invention provides pharmaceutical compositions comprising the chemical compound of the invention or a  
30 pharmaceutically acceptable salt or derivative thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being

compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical composition of the invention may be administered by any convenient route which suite the desired therapy. Preferred routes of administration include oral administration, in particular in tablet, in capsule, in dragé, in powder, or in liquid form, and parenteral administration, in particular cutaneous, subcutaneous, intramuscular, or intravenous injection. The pharmaceutical composition may be prepared by the skilled person using standard and conventional techniques appropriate to the desired formulation. When desired, compositions adapted to give sustained release of the active ingredient may be employed.

The actual dosage depend on the nature and severity of the disease being treated, and is within the discretion of the physician, and may be varied by titration of the dosage to the particular circumstances of this invention to produce the desired therapeutic effect. However, it is presently contemplated that pharmaceutical compositions containing of from about 0.1 to about 500 mg of active ingredient per individual dose, preferably of from about 1 to about 100 mg, most preferred of from about 1 to about 10 mg, are suitable for therapeutic treatments.

The active ingredient may be administered in one or several doses per day. A satisfactory result can, in certain instances, be obtained at a dosage as low as 0.1  $\mu\text{g/kg}$  i.v. and 1  $\mu\text{g/kg}$  p.o. The upper limit of the dosage range is presently considered to be about 10 mg/kg i.v. and 100 mg/kg p.o. Preferred ranges are from about 0.1  $\mu\text{g/kg}$  to about 10 mg/kg i.v., and from about 1  $\mu\text{g/kg}$  to about 100 mg/kg p.o.

In a preferred embodiment, the pharmaceutical composition of the invention comprises a chemical compound which is an imidazole derivative, in particular Clotrimazole, Miconazole, Ketonazole, Econazole, Butoconazole, Oxiconazole, Sulconazole, or Tioconazole.

In another preferred embodiment, the pharmaceutical composition of the invention comprises a chemical compound which is a nitroimidazole derivative, in particular Metronidazole, Tinidazole, Nimorazole, Ornidazole, or Benznidazole.

In yet another preferred embodiment, the pharmaceutical composition of the invention comprises a chemical compound which is a triazole derivative, in particular Fluconazole, Tercolazole, or Itraconazole.



In a further preferred embodiment, the pharmaceutical composition of the invention comprises a chemical compound which is a metabolite of Clotrimazole, in particular 2-chlorophenyl-4-hydroxy-phenyl-phenyl-methane, 2-chlorophenyl-bis-phenyl-methane, or 2-chlorophenyl-bis-phenyl-methanol.

5

### Method of Treatment

The  $IK_{Ca}$  inhibitory compounds of the invention are useful as immune modulating agents, i.e. agents capable of regulating the immune system, and may be used in a method of for reducing or inhibiting undesired immunoregulatory actions.

10

Therefore, in a separate aspect, the invention relates to a method of treatment or alleviation of diseases, disorders or conditions relating to immune dysfunction in a living body, said method comprising administering to said living body an effective amount of a chemical compound having  $IK_{Ca}$  inhibitory activity.

15

### EXAMPLES

The invention is further illustrated with reference to the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

#### 20 EXAMPLE 1

**Isolation of a cDNA encoding human placenta  $Ca^{2+}$ -activated, intermediate conductance potassium channel protein**

The full length coding sequence of a cDNA encoding human placenta  $Ca^{2+}$ -activated, intermediate conductance potassium channel protein (SEQ ID NO: 2) is  
25 radio labelled by random priming and used as a hybridisation probe to screen a human placenta cDNA library under hybridisation conditions of 1 M NaCl, 1% SDS and 50% formamide at 42°C. Hybridisation wash conditions are 55°C, 0.2 x SSC and 0.1% SDS. Positively hybridising clones are purified and the nucleotide and predicted amino acid sequence are determined.

30

**EXAMPLE 2****Assay for DNA encoding  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein**

The presence of DNA encoding human placenta  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein was determined by transfecting mammalian cells with a cDNA preparation and using the membrane patch clamp technique [Hamill, O.P., et al., *Pflügers Arch.* 1981 351 85-100] or using the Fluorescence Image Plate Reader (FLIPR) assay.

A cDNA encoding the human placenta  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein was identified by a BLAST search of the expressed sequence tag (EST) database using the query sequence (51 Amino acids):

LGHRRALFEKRKRLSDYALIFGMFGIVVMVIETELSWGLYSKDSMFSLALC

(SEQ ID NO: 3),

and allowing for mismatches.

A BLAST search retrieved the GenBank Entry No. N56819, a cDNA encoding the entire  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein.

HEK293 or CHO tissue culture cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FCS (foetal calf serum) at 37°C in 5%  $\text{CO}_2$ . One day prior to transfection,  $10^6$  cells were plated in a cell culture T25 flask. The following day, cells were transfected using lipofection (20  $\mu\text{L}$  Lipofectamin™, Life Technologies, with 2.5  $\mu\text{g}$  of the plasmid pNS2Z\_hIK2 in a total volume of 540  $\mu\text{L}$ ).

Two different plasmids were used for transfection. Cells prepared for the electrophysiological screening were transfected with pNS2Z\_hIK2, which besides coding for the human placenta  $\text{Ca}^{2+}$ -activated intermediate conductance potassium channel protein also codes for the green fluorescent protein EGFP. Cells prepared for the FLIPR assay were transfected with pNS1Z\_hIK2, which is an analogue to pNS2Z\_hIK2 but without the cDNA encoding EGFP. The lipofection mixture was overlaid on the cells and incubated at 37°C for 5 hours. The cells were then rinsed with regular media and plated either to 30 mm culture dishes (electrophysiological assay) or to 96-well microtiter plates (FLIPR assay).

18-48 hours after transfection cells were assayed for the presence of  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein.

Transfected HEK293 cells were assayed for the presence of  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein by a fluometric technique based on the membrane potential sensitive dye DIBAC<sub>4</sub>(3). After transfection, cells were washed twice with a 5  $\mu\text{M}$  DIBAC<sub>4</sub>(3)/FLIPR buffer solution (100  $\mu\text{l}$  in each well). The FLIPR buffer solution contained in mM: 145 NaCl, 1 KCl, 1  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , 10 HEPES, 10 glucose and with pH adjusted to 7.4. After the cell wash 180  $\mu\text{l}$  DIBAC<sub>4</sub>(3)/FLIPR buffer solution was added to each well and the microtiter plate was equilibrated at 35°C for 20-30 min. A drugplate containing Ionomycin and Thapsigargin was made 10x concentrated and was also equilibrated at 35°C before starting the experiment.

The FLIPR was programmed to do a sample reading every 20 sec. for a total period of 10 min. The assay was started with a pre-run for 1 min, followed by a simultaneous addition of 20  $\mu\text{l}$  "drug" to all 96 wells. Addition of Ionomycin and Thapsigargin both result in an increase in the intracellular  $\text{Ca}^{2+}$  concentration, which in turn activated the intermediate conductance potassium channels. This activation was observed as a decrease in the fluorescent signal which correlates to a membrane hyperpolarization.

### EXAMPLE 3

#### Assays for DNA encoding $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein.

HEK293 or CHO tissue culture cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FCS (foetal calf serum) at 37° C in 5%  $\text{CO}_2$ . One day prior to transfection,  $10^6$  cells were plated in a cell culture T25 flask. The following day, cells were transfected using lipofection (20  $\mu\text{L}$  Lipofectamin™, Life Technologies, with 2.5  $\mu\text{g}$  of the plasmid pNS2Z\_hIK2 in a total volume of 540  $\mu\text{L}$ ). The lipofection mixture was overlaid on the cells and incubated at 37°C for 5 hours.

The cells were then rinsed with regular media and plated to a 30 mm culture dish. 18-48 hours after, transfected cells were assayed for the presence of  $\text{Ca}^{2+}$ .

activated, intermediate conductance potassium channel protein by electrophysiological measurements.

The presence of DNA encoding human placenta  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channel protein was determined by transfecting  
5 mammalian cells with a cDNA preparation and by using the patch clamp technique (see Hamill *OP et al.*, Pflügers Arch. 1981 351 85-100).

Whole cell currents were recorded using a pipette solution of 144 mM KCl, 1 mM EGTA, 9 mM NTA, 1.085 mM  $\text{CaCl}_2$ , 5.54 mM  $\text{MgCl}_2$ , and 10 mM HEPES (pH 7.2) and a bath solution of 144 mM KCl, 2 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 0.01% (w/v) BSA  
10 and 10 mM HEPES (pH = 7.4). Current traces were recorded from cells containing human  $\text{Ca}^{2+}$ -activated, intermediate conductance potassium channels after application of voltage ramps (- 100 mV to + 100 mV, 200 ms duration).

Clotrimazole sensitivity of the expressed channels was determined by addition of 1  $\mu\text{M}$  Clotrimazole to the bath solution. Application of Clotrimazole resulted  
15 in an inhibition of the  $\text{Ca}^{2+}$ -activated potassium current which was reversed by washout of Clotrimazole from the bath solution.

An  $\text{IC}_{50}$  value of 153 nM for Clotrimazole was calculated from the kinetics of the block.

#### 20 EXAMPLE 4

##### Inhibition of T Cell Proliferation

The chemical compounds used according to the invention prevent immunological proliferation by selective inhibition of the  $\text{Ca}^{2+}$ -activated K-channels in T- and B-lymphocytes. This effect may be verified using various proliferation assays.  
25 In this experiment the proliferative assay described by Ødum *et al.* [Ødum N, Kanner S B, Ledbetter J A, & Svejgaard A; J. Immunol. 1993 150 (12) 5289-5298] was used.

The chemical compounds representative of the invention tested in this experiment are Nitrendipine, a derivative of the 1,4-dihydropyridine-3,5-dicarboxylic acid, and the imidazole derivative Clotrimazole.

30 Assays were performed in culture medium (RPMI 1640; available from Gibco, Grand Island, NY) supplemented with 10% pooled human serum, 2 mM L-glutamine, 100  $\mu\text{g/ml}$  penicillin, and 100  $\mu\text{g/ml}$  streptomycin (available from Novo

Nordisk, Copenhagen, Denmark) in 96-well round bottom tissue culture plates (available from Nunc, Roskilde, Denmark) with a final volume of 200  $\mu$ l.

T cells were preincubated for three hours with the test compounds before addition of antigen (crude *Candida albicans* extract, 10  $\mu$ g/ml). T cells were cultured at  $5 \times 10^4$  cells/well for 144 hours. Twelve hours before harvest, [ $^3$ H]thymidine (1 x Ci/well) was added. The cells were harvested onto glass fibre filters, and the [ $^3$ H]thymidine incorporation was measured in a scintillation counter. The results were expressed as mean counts per minute (cpm) from triplicate cultures.

The results are presented in Table 1, below.

10

**Table 1****Inhibition of T Cell Proliferation**

	T Cell Proliferation (cpm x 10 <sup>-3</sup> )			
	Medium	Antigen		
	Solvent	1 $\mu$ M	5 $\mu$ M	10 $\mu$ M
Clotrimazole	0.2	5.8	4.2	1.8
Nitrendipine	0.2	5.6	3.8	4.0

15

These results show that the number of T cells decreases in the presence of increasing concentrations of the chemical compound of the invention, and support the fact that the chemical compounds of the invention inhibit the antigen induced T cell proliferation and thus are useful for the reduction or inhibition of undesired immunoregulatory actions.

## SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 1284 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..1284

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATG GGC GGG GAT CTG GTG CTT GGC CTG GGG GCC TTG AGA CGC CGA AAG 48  
Met Gly Gly Asp Leu Val Leu Gly Leu Gly Ala Leu Arg Arg Arg Lys  
20 1 5 10 15

CGC TTG CTG GAG CAG GAG AAG TCT CTG GCC GGC TGG GCA CTG GTG CTG 96  
Arg Leu Leu Glu Gln Glu Lys Ser Leu Ala Gly Trp Ala Leu Val Leu  
20 25 30

25 GCA GGA ACT GGC ATT GGA CTC ATG GTG CTG CAT GCA GAG ATG CTG TGG 144  
Ala Gly Thr Gly Ile Gly Leu Met Val Leu His Ala Glu Met Leu Trp  
35 40 45

30 TTC GGG GGG TGC TCG TGG GCG CTC TAC CTG TTC CTG GTT AAA TGC ACG 192  
Phe Gly Gly Cys Ser Trp Ala Leu Tyr Leu Phe Leu Val Lys Cys Thr  
50 55 60

ATC AGC ATT TCC ACC TTC TTA CTC CTC TGC CTC ATC GTG GCC TTT CAT 240  
35 Ile Ser Ile Ser Thr Phe Leu Leu Leu Cys Leu Ile Val Ala Phe His  
65 70 75 80

GCC AAA GAG GTC CAG CTG TTC ATG ACC GAC AAC GGG CTG CGG GAC TGG 288  
Ala Lys Glu Val Gln Leu Phe Met Thr Asp Asn Gly Leu Arg Asp Trp  
40 85 90 95

CGC GTG GCG CTG ACC GGG CGG CAG GCG GCG CAG ATC GTG CTG GAG CTG 336  
Arg Val Ala Leu Thr Gly Arg Gln Ala Ala Gln Ile Val Leu Glu Leu  
100 105 110

	GTG GTG TGT GGG CTG CAC CCG GCG CCC GTG CGG GGC CCG CCG TGC GTG Val Val Cys Gly Leu His Pro Ala Pro Val Arg Gly Pro Pro Cys Val	384
	115 120 125	
5	CAG GAT TTA GGG GCG CCG CTG ACC TCC CCG CAG CCC TGG CCG GGA TTC Gln Asp Leu Gly Ala Pro Leu Thr Ser Pro Gln Pro Trp Pro Gly Phe	432
	130 135 140	
	CTG GGC CAA GGG GAA GCG CTG CTG TCC CTG GCC ATG CTG CTG CGT CTC Leu Gly Gln Gly Glu Ala Leu Leu Ser Leu Ala Met Leu Leu Arg Leu	480
10	145 150 155 160	
	TAC CTG GTG CCC CGC GCC GTG CTC CTG CGC AGC GGC GTC CTG CTC AAC Tyr Leu Val Pro Arg Ala Val Leu Leu Arg Ser Gly Val Leu Leu Asn	528
15	165 170 175	
	GCT TCC TAC CGC AGC ATC GGC GCT CTC AAT CAA GTC CGC TTC CGC CAC Ala Ser Tyr Arg Ser Ile Gly Ala Leu Asn Gln Val Arg Phe Arg His	576
	180 185 190	
20	TGG TTC GTG GCC AAG CTT TAC ATG AAC ACG CAC CCT GGC CGC CTG CTG Trp Phe Val Ala Lys Leu Tyr Met Asn Thr His Pro Gly Arg Leu Leu	624
	195 200 205	
25	CTC GGC CTC ACG CTT GGC CTC TGG CTG ACC ACC GCC TGG GTG CTG TCC Leu Gly Leu Thr Leu Gly Leu Trp Leu Thr Thr Ala Trp Val Leu Ser	672
	210 215 220	
	GTG GCC GAG AGG CAG GCT GTT AAT GCC ACT GGC CAC CTT TCA GAC ACA Val Ala Glu Arg Gln Ala Val Asn Ala Thr Gly His Leu Ser Asp Thr	720
30	225 230 235 240	
	CTT TGG CTG ATT CCC ATC ACA TTC CTG ACC ATC GGC TAT GGT GAC GTG Leu Trp Leu Ile Pro Ile Thr Phe Leu Thr Ile Gly Tyr Gly Asp Val	768
35	245 250 255	
	GTG CCG GGC ACC ATG TGG GGC AAG ATC GTC TGC CTG TGC ACT GGA GTC Val Pro Gly Thr Met Trp Gly Lys Ile Val Cys Leu Cys Thr Gly Val	816
	260 265 270	
40	ATG GGT GTC TGC TGC ACA GCC CTG CTG GTG GCC GTG GTG GCC CGG AAG Met Gly Val Cys Cys Thr Ala Leu Leu Val Ala Val Val Ala Arg Lys	864
	275 280 285	

	CTG GAG TTT AAC AAG GCA GAG AAG CAC GTG CAC AAC TTC ATG ATG GAT	912
	Leu Glu Phe Asn Lys Ala Glu Lys His Val His Asn Phe Met Met Asp	
	290 295 300	
5	ATC CAG TAT ACC AAA GAG ATG AAG GAG TCC GCT GCC CGA GTG CTA CAA	960
	Ile Gln Tyr Thr Lys Glu Met Lys Glu Ser Ala Ala Arg Val Leu Gln	
	305 310 315 320	
	GAA GCC TGG ATG TTC TAC AAA CAT ACT CGC AGG AAG GAG TCT CAT GCT	1008
10	Glu Ala Trp Met Phe Tyr Lys His Thr Arg Arg Lys Glu Ser His Ala	
	325 330 335	
	GCC CGC AGG CAT CAG CGC AAG CTG CTG GCC GCC ATC AAC GCG TTC CGC	1056
	Ala Arg Arg His Gln Arg Lys Leu Leu Ala Ala Ile Asn Ala Phe Arg	
15	340 345 350	
	CAG GTG CGG CTG AAA CAC CGG AAG CTC CGG GAA CAA GTG AAC TCC ATG	1104
	Gln Val Arg Leu Lys His Arg Lys Leu Arg Glu Gln Val Asn Ser Met	
	355 360 365	
20	GTG GAC ATC TCC AAG ATG CAC ATG ATC CTG TAT GAC CTG CAG CAG AAT	1152
	Val Asp Ile Ser Lys Met His Met Ile Leu Tyr Asp Leu Gln Gln Asn	
	370 375 380	
25	CTG AGC AGC TCA CAC CGG GCC CTG GAG AAA CAG ATT GAC ACG CTG GCG	1200
	Leu Ser Ser Ser His Arg Ala Leu Glu Lys Gln Ile Asp Thr Leu Ala	
	385 390 395 400	
	GGG AAG CTG GAT GCC CTG ACT GAG CTG CTT AGC ACT GCC CTG GGG CCG	1248
30	Gly Lys Leu Asp Ala Leu Thr Glu Leu Leu Ser Thr Ala Leu Gly Pro	
	405 410 415	
	AGG CAG CTT CCA GAA CCC AGC CAG CAG TCC AAG TAG	1284
	Arg Gln Leu Pro Glu Pro Ser Gln Gln Ser Lys *	
35	420 425	

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 428 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Gly Gly Asp Leu Val Leu Gly Leu Gly Ala Leu Arg Arg Arg Lys  
 1 5 10 15  
 5 Arg Leu Leu Glu Gln Glu Lys Ser Leu Ala Gly Trp Ala Leu Val Leu  
 20 25 30  
 Ala Gly Thr Gly Ile Gly Leu Met Val Leu His Ala Glu Met Leu Trp  
 10 35 40 45  
 Phe Gly Gly Cys Ser Trp Ala Leu Tyr Leu Phe Leu Val Lys Cys Thr  
 50 55 60  
 15 Ile Ser Ile Ser Thr Phe Leu Leu Leu Cys Leu Ile Val Ala Phe His  
 65 70 75 80  
 Ala Lys Glu Val Gln Leu Phe Met Thr Asp Asn Gly Leu Arg Asp Trp  
 85 90 95  
 20 Arg Val Ala Leu Thr Gly Arg Gln Ala Ala Gln Ile Val Leu Glu Leu  
 100 105 110  
 Val Val Cys Gly Leu His Pro Ala Pro Val Arg Gly Pro Pro Cys Val  
 25 115 120 125  
 Gln Asp Leu Gly Ala Pro Leu Thr Ser Pro Gln Pro Trp Pro Gly Phe  
 130 135 140  
 30 Leu Gly Gln Gly Glu Ala Leu Leu Ser Leu Ala Met Leu Leu Arg Leu  
 145 150 155 160  
 Tyr Leu Val Pro Arg Ala Val Leu Leu Arg Ser Gly Val Leu Leu Asn  
 165 170 175  
 35 Ala Ser Tyr Arg Ser Ile Gly Ala Leu Asn Gln Val Arg Phe Arg His  
 180 185 190  
 Trp Phe Val Ala Lys Leu Tyr Met Asn Thr His Pro Gly Arg Leu Leu  
 40 195 200 205  
 Leu Gly Leu Thr Leu Gly Leu Trp Leu Thr Thr Ala Trp Val Leu Ser  
 210 215 220

Val Ala Glu Arg Gln Ala Val Asn Ala Thr Gly His Leu Ser Asp Thr  
 225 230 235 240  
 Leu Trp Leu Ile Pro Ile Thr Phe Leu Thr Ile Gly Tyr Gly Asp Val  
 5 245 250 255  
 Val Pro Gly Thr Met Trp Gly Lys Ile Val Cys Leu Cys Thr Gly Val  
 260 265 270  
 10 Met Gly Val Cys Cys Thr Ala Leu Leu Val Ala Val Val Ala Arg Lys  
 275 280 285  
 Leu Glu Phe Asn Lys Ala Glu Lys His Val His Asn Phe Met Met Asp  
 290 295 300  
 15 Ile Gln Tyr Thr Lys Glu Met Lys Glu Ser Ala Ala Arg Val Leu Gln  
 305 310 315 320  
 Glu Ala Trp Met Phe Tyr Lys His Thr Arg Arg Lys Glu Ser His Ala  
 20 325 330 335  
 Ala Arg Arg His Gln Arg Lys Leu Leu Ala Ala Ile Asn Ala Phe Arg  
 340 345 350  
 25 Gln Val Arg Leu Lys His Arg Lys Leu Arg Glu Gln Val Asn Ser Met  
 355 360 365  
 Val Asp Ile Ser Lys Met His Met Ile Leu Tyr Asp Leu Gln Gln Asn  
 370 375 380  
 30 Leu Ser Ser Ser His Arg Ala Leu Glu Lys Gln Ile Asp Thr Leu Ala  
 385 390 395 400  
 Gly Lys Leu Asp Ala Leu Thr Glu Leu Leu Ser Thr Ala Leu Gly Pro  
 35 405 410 415  
 Arg Gln Leu Pro Glu Pro Ser Gln Gln Ser Lys \*  
 420 425

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 amino acids

(B) TYPE: amino acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Leu Gly His Arg Arg Ala Leu Phe Glu Lys Arg Lys Arg Leu Ser Asp  
1 5 10 15

15

Tyr Ala Leu Ile Phe Gly Met Phe Gly Ile Val Val Met Val Ile Glu  
20 25 30

20

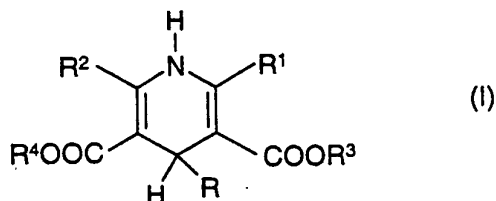
Thr Glu Leu Ser Trp Gly Leu Tyr Ser Lys Asp Ser Met Phe Ser Leu  
35 40 45

Ala Leu Cys

50

## CLAIMS

1. Use of a chemical compound having  $IK_{Ca}$  inhibitory activity for the manufacture of a medicament for the treatment or alleviation of diseases, disorders or conditions relating to immune dysfunction.
2. The use according to claim 1, in which the chemical compound is a symmetric or asymmetric derivative of 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general formula



wherein

R represents an alkyl group or a cycloalkyl group;

or R represents a mono- or polycyclic aryl group, which aryl group may be substituted one or more times with substituents selected from the group consisting of halogen, trifluoromethyl ( $-CF_3$ ), nitro ( $-NO_2$ ), cyano ( $-CN$ ), azido ( $-N_3$ ), a group of the formula  $-S(O)_n$ -alkyl,  $-S(O)_n$ -NH-alkyl, or  $-S(O)_n$ -N-(alkyl) $_2$ , in which n has a value of 0, 1 or 2, an alkyl group, a cycloalkyl group, an alkoxy group, a trifluoromethyl-oxy group ( $-OCF_3$ ), a carboxy group ( $-COOH$ ), a group of the formula  $-COO$ -alkyl, a carbamoyl group ( $-CONH_2$ ), and a group of the formula  $-CONH$ -alkyl or  $-CON$ (alkyl) $_2$ ;

or R represents a mono- or poly-heterocyclic group, which heterocyclic group may be substituted one or more times with alkyl, alkoxy, a carboxy group ( $-COOH$ ), a group of the formula  $-COO$ -alkyl, and/or a group of the formula  $-COO$ -phenyl;

and R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup>, independent of each another, represents hydrogen, an alkyl group, a cycloalkyl group, an alkenyl group, an alkynyl group, an alkoxy group, a phenyl group, a phenyl-alkyl group, a furanyl group, a furanyl-alkyl group, a pyridyl group, or a pyridyl-alkyl group;

or a pharmaceutically acceptable acid addition salt thereof.

3. The use according to claim 2, in which R represents a C<sub>3-7</sub>-cycloalkyl group, in particular cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl;

or R represents a phenyl group, which phenyl group may be substituted one or more times with substituents selected from the group consisting of halogen, trifluoromethyl (-CF<sub>3</sub>), nitro (-NO<sub>2</sub>), cyano (-CN), azido (-N<sub>3</sub>), a group of the formula -S(O)<sub>n</sub>-alkyl, -S(O)<sub>n</sub>-NH-alkyl, or -S(O)<sub>n</sub>-N-(alkyl)<sub>2</sub>, in which n has a value of 0, 1 or 2, an alkyl group, a cycloalkyl group, an alkoxy group, a trifluoromethyloxy group (-OCF<sub>3</sub>), a carboxy group (-COOH), a group of the formula -COO-alkyl, a carbamoyl group (-CONH<sub>2</sub>), and a group of the formula -CONH-alkyl or -CON(alkyl)<sub>2</sub>;

or R represents a pyridyl group or a dihydro-pyridyl group, which groups may be monosubstituted with a group of the formula -COO-alkyl, or a group of the formula -COO-phenyl.

4. The use according to claim 3, in which R represents a 2-nitrophenyl group, a 3-nitrophenyl group, a 4-nitrophenyl group, a 2-trifluoromethylphenyl group, a 3-trifluoromethylphenyl group, a 4-trifluoromethylphenyl group, a 2-cyanophenyl group, a 3-cyanophenyl group, or a 4-cyanophenyl group;

or R represents a 2-pyridyl, a 3-pyridyl or a 4-pyridyl group, a 1,2-, 1,4- or 1,6-dihydro-2-pyridyl, a 1,2-, 1,4- or 1,6-dihydro-3-pyridyl, or a 1,2- or 1,4-dihydro-4-pyridyl group, which pyridyl or dihydropyridyl groups may be monosubstituted with C<sub>1-6</sub>-alkyl, a group of the formula -COO-C<sub>1-6</sub>-alkyl, or a group of the formula -COO-phenyl.

5. The use according to claim 2, in which  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$ , independent of each another, represents  $C_{1-6}$ -alkyl group, in particular methyl, ethyl, propyl, isopropyl, butyl, or isobutyl; a  $C_{3-7}$ -cycloalkyl group, in particular cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl; a furanyl group, in particular a 2-furanyl- $C_{1-6}$ -alkyl group, preferably 2-furanyl-methyl.  
5
6. The use according to claim 2, in which R represents cyclohexyl, and  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$ , independent of each another, represent methyl or ethyl.  
10
7. The use according to claim 2, in which R represents phenyl, and  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$ , independent of each another, represent methyl or ethyl.
8. The use according to claim 2, in which R represents 4-nitrophenyl, and  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$ , independent of each another, represent methyl or ethyl.  
15
9. The use according to claim 2, in which R represents 3-pyridyl, and  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$ , independent of each another, represent methyl or ethyl.
- 20 10. The use according to claim 2, in which R represents 3-pyridyl,  $R^1$  and  $R^2$  independent of each another represent methyl or ethyl,  $R^3$  represents isopropyl, and  $R^4$  represents 2-furanyl-methyl.
11. The use according to claim 2, in which the chemical compound is an asymmetric derivative of the 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general formula (I).  
25
12. The use according to claim 11, in which the chemical compound is an asymmetric  $C_{1-6}$ -alkyl derivative of the 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general formula (I).  
30
13. The use according to claim 12, in which the chemical compound is

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;

5 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester (Nitrendipine);

10 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
ethyl methyl ester;

15 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;

20 1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid isopropyl methyl ester;

25 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid propyl methyl ester;

30 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

5 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

10 1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

15 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

20 1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

25 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid isopropyl methyl ester;

30 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid ethyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid propyl methyl ester;



1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid ethyl  
methyl ester;

5 1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid propyl  
methyl ester; or

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid  
isopropyl methyl ester.

10 14. The use according to claim 2, in which the chemical compound is a symmetric  
derivative of 1,4-dihydropyridine-3,5-dicarboxylic acid represented by the general  
formula (I).

15 15. The use according to claim 14, in which the chemical compound is a symmetric  
C<sub>1-6</sub>-alkyl derivative of the 1,4-dihydropyridine-3,5-dicarboxylic acid represented  
by the general formula (I).

16. The use according to claim 15, in which the chemical compound is

20 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester (Nifedipine);

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;

25 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
dimethyl ester;

30 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylic acid  
diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic  
acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-trifluorophenyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

5 1,4-dihydro-2,6-dimethyl-4-(3-trifluorophenyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

10 1,4-dihydro-2,6-dimethyl-4-(4-trifluorophenyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-cyanophenyl)pyridine-3,5-dicarboxylic acid diethyl ester;

15 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)pyridine-3,5-dicarboxylic acid diethyl ester;

20 1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-cyanophenyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

25 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

30 1,4-dihydro-2,6-dimethyl-4-(3-pyridyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid dimethyl ester;

1,4-dihydro-2,6-dimethyl-4-(4-pyridyl)pyridine-3,5-dicarboxylic acid diethyl ester;

1,4-dihydro-2,6-dimethyl-4-cyclohexylpyridine-3,5-dicarboxylic acid dimethyl ester; or

5        1,4-dihydro-2,6-dimethyl-4-cyclohexylpyridine-3,5-dicarboxylic acid diethyl ester.

17. The use according to claim 1, in which the chemical compound is an imidazole derivative selected from the group consisting of

- 10        1-[(2-chlorophenyl)-diphenyl-methyl]-1H-imidazole (Clotrimazole);  
1-[(3-chlorophenyl)-diphenyl-methyl]-1H-imidazole;  
1-[(4-chlorophenyl)-diphenyl-methyl]-1H-imidazole;  
1-[(2-chlorophenyl)-(4-hydroxyphenyl)-phenyl-methyl]-1H-imidazole;  
1-[(3-chlorophenyl)-(4-hydroxyphenyl)-phenyl-methyl]-1H-imidazole;  
15        1-[(4-chlorophenyl)-(4-hydroxyphenyl)-phenyl-methyl]-1H-imidazole;  
1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]-1H-imidazole (Miconazole);  
1-Acetyl-4[4-[(2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4yl)methoxy]phenyl]piperazine (Ketoconazole);  
20        1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole (Econazole);  
1-[4-(4-chlorophenyl)-2-(2,6-dichlorophenylthio)butyl]imidazole mononitrate (Butoconazole);  
2',4'-dichloro-2-imidazol-1-ylacetophenone-(Z)-O-(2,4-dichlorobenzyl)oxime mononitrate (Oxiconazole);  
25        1-[2,4-dichloro-β-(4-chlorobenzyl)thiophenethyl]imidazole nitrate (Sulconazole); and  
1-[2-[(2-chloro-3-thienyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole (Thioconazole).

30

18. The use according to claim 1, in which the chemical compound is a triazole derivative selected from the group consisting of

2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol (Fluconazole);

1-{4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-phenyl}-4-isopropylpiperazine (Terconazole);

(±)-2-sec-butyl-4-[4-(4-{[(2R\*,4S\*)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-ylmethoxy]phenyl}-piperazin-1-yl)phenyl]-2,4-dihydro-1,2,4-triazol-3-one (Itraconazole).

19. The use according to claim 1, in which the chemical compound is a nitroimidazole derivative selected from the group consisting of

2-methyl-5-nitroimidazole-1-ethanol (Metronidazole);

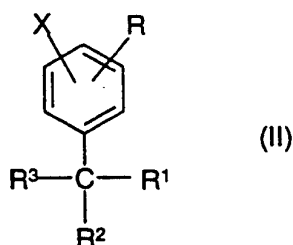
1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole (Tinidazole);

4-[2-(5-nitroimidazol-1-yl)ethyl]morpholine (Nimorazole);

1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol (Omidazole); and

N-benzyl-2-(2-nitroimidazol-1-yl)acetamide (Benznidazole).

20. The use according to claim 1, in which the chemical compound is a derivative or metabolite of Clotrimazole characterised by the following general formula



wherein

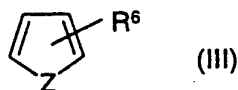
X represents halogen, a trifluoromethyl group, a nitro group, or a cyano group;

R represents hydrogen, halogen, hydroxy, an alkyl group, a cycloalkyl group, an alkoxy group, or an alkyloxy group;

R<sup>1</sup> represents hydrogen, or a phenyl group, which phenyl group may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy;

5 R<sup>2</sup> represents hydrogen, hydroxyl, alkyl, alkoxy;

R<sup>3</sup> represents a group of the formula -Y-CH<sub>2</sub>-R<sup>5</sup>, wherein Y represents oxygen (-O-) or sulphur (-S-); a group of the formula =NO-CH<sub>2</sub>R<sup>5</sup>; a group of the formula -O-phenyl-CH=CH<sub>2</sub>; a group of the formula -CH<sub>2</sub>-CH(CH<sub>3</sub>)-S-phenyl, which phenyl  
10 may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; or a phenyl group, which phenyl may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; and wherein R<sup>5</sup> represents an ethenyl group (CH<sub>2</sub>=CH-); a phenyl group, which phenyl may be substituted one or more times  
15 with substituents selected from the group consisting of halogen and hydroxy; a phenyl-S-phenyl group, a group of the formula CH<sub>2</sub>-O-phenyl, which phenyl may be substituted one or more times with substituents selected from the group consisting of halogen and hydroxy; or a group of the formula



20 wherein Z represents S, O or N;  
and R<sup>6</sup> represents hydrogen, halogen or hydroxy;

or a pharmaceutically acceptable acid addition salt thereof.

25 21. The use according to claim 20, in which the derivative or metabolite of Clotrimazole is

2-chlorophenyl-4-hydroxyphenyl-phenyl-methane;

2-chlorophenyl-bis-phenyl-methane;

2-chlorophenyl-bis-phenyl-methanol;

30 3-(1-[2,4-dichlorophenyl]-ethoxymethyl)-2-chlorothiophene;

O-(2,4-dichlorobenzyl)-2,4-dichloroacetophenone oxime;

1-(2,4-dichloro)-1-(4-(phenylthio)benzyloxy)ethane;  
1-(2,4-dichlorophenyl)-1-(allyloxy)ethane;  
1-(2,4-dichlorophenyl)-1-(4-chlorobenzylthio)ethane;  
1-(2,4-dichlorophenyl)-1-(2,4-dichlorobenzyloxy)ethane;  
5 1-(2,4-dichlorophenyl)ethyl-2,6-dichlorobenzyl ether;  
1-(2-[4-chlorophenoxy]ethyloxy)-1-(2,4-dichlorophenyl)propene;  
1-(2,4-dichlorophenyl)-ethyl-(4-chlorophenyl)methyl ether;  
3-chlorobenzyl-2-vinylphenyl ether; and  
1-(4-chlorophenyl)-3-(2,6-dichlorophenylthio)butane.

10

22. A pharmaceutical composition for use in the treatment or alleviation of diseases, disorders or conditions relating to immune dysfunction, which pharmaceutical composition comprises an effective amount of a chemical compound having  $IK_{Ca}$  inhibitory activity.

15

23. A method for treatment or alleviation of diseases, disorders or conditions relating to immune dysfunction in a living body, said method comprising administering to said living body an effective amount of a chemical compound having  $IK_{Ca}$  inhibitory activity.

20

24. A method of screening a chemical compound for inhibitory activity on an intermediate conductance  $Ca^{2+}$  activated potassium channel ( $IK_{Ca}$ ), which method comprises the steps of

25

- (i) subjecting an  $IK_{Ca}$  containing cell to the action of the chemical compound, and
- (ii) monitoring the membrane potential of the  $IK_{Ca}$  containing cell.

30

25. The method according to claim 24, wherein the  $IK_{Ca}$  of the  $IK_{Ca}$  containing cell is an ion channel that is endogenous to the cell, e.g. a human epithelial-like cell line such as a HeLa cell (epitheloid carcinoma, cervix, human), a T- or B-lymphocyte, a cell of epithelial origin, a cell of endothelial origin, or a blood cell.

26. The method according to claim 24, wherein the  $IK_{Ca}$  of the  $IK_{Ca}$  containing cell is an ion channel that is exogenous to the cell, e.g. a human embryonic kidney cell (a HEK cell), a HEK 293 cell, a Chinese hamster ovary (CHO) cell, or a *Xenopus laevis* oocyte (XLO) cell.
- 5
27. The method according to claim 24, wherein the  $IK_{Ca}$  is isolated from salivary glands, from lung tissue, from tracheal tissue, from placenta tissue, from pancreas tissue, from lymphocytes, from colon tissue, from kidney tissue, from thymus tissue, from bone marrow, from prostate tissue, from stomach tissue,  
10 from liver tissue, from foetal liver tissue, from mammary glands, from small intestine tissue, from spleen tissue, or from lymph node tissue.
28. The method according to claim 24, wherein the  $IK_{Ca}$  is encoded by the DNA sequence presented as SEQ ID NO: 1, or a sequence analogous hereto.
- 15
29. The method according to any of claims 24-28, wherein monitoring the membrane potential of the  $IK_{Ca}$  containing cell is accomplished by patch clamp technology.
30. The method according to any of claims 24-28, wherein,  
20 in step (i), the  $IK_{Ca}$  containing cell is mixed with a membrane potential indicating agent; and  
in step (ii), monitoring the membrane potential of the  $IK_{Ca}$  containing cell is accomplished by spectrophotometry.
- 25 31. The method according to claim 30, wherein the membrane potential indicating agent is a fluorescent agent, in particular DIBAC<sub>4</sub>(3), DiOC5(3), or DiOC2(3).
32. The method according to either of claims 30-31, wherein the membrane potential of the  $IK_{Ca}$  containing cell is accomplished using a Fluorescence Image Plate  
30 Reader (a FLIPR assay).